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Masahiro Horiuchi[*,#], Gabriella M.K. Rossetti[#], Samuel J. Oliver

A healthy diet combined with increased physical activity levels may protect against cerebrovascular dysfunction and cognitive impairments (Gorelick et al., 2011). Compared with the number of studies on the effects of physical activity, studies on how diet affects cerebral blood flow (CBF) regulation and cognitive function are relatively limited. Here, we focus on the potential role of dietary nitrate supplementation in CBF regulation and cognitive function under normoxic and hypoxic conditions.

The precise regulation of CBF is critical for maintaining a constant supply of nutrients and oxygen to the brain because of the high metabolic rate of brain tissue. Despite accounting for only 2% of the total body weight the brain accounts for 20% of the body's oxygen and calorie consumption. CBF is normally well controlled at a constant level over a wide range of perfusion pressures via cerebral autoregulation (CA, **Figure 1A**; Tzeng and Ainslie, 2014). Resting CBF gradually decreases with healthy aging, and to a greater extent in individuals who develop cognitive impairment and dementia (Gorelick et al., 2011). Dynamic CA is impaired in several clinical conditions, which can also be characterized by hypoxia, such as stroke, anemia, and Alzheimer's disease (Kuwabara et al., 2002), and is implicated alongside reduced CBF in the development of cognitive dysfunction (Tzeng and Ainslie, 2014). Moreover, acute hypoxia in otherwise healthy adults has also been shown to impair dynamic CA (Horiuchi et al., 2020). Thus, investigation of methods to maintain CBF and protect dynamic CA is important in an aging society, in clinical settings, and in those working and performing in low oxygen environments e.g., soldiers and mountaineers.

Potential role of nitric oxide (NO) in CBF regulation and neurovascular coupling: Although the physiological mechanisms responsible for the control of CBF via CA are complex, NO is a primary regulator of vascular tone throughout the human body (Tzeng and Ainslie, 2014). In vascular smooth muscle cells, NO interacts with the haem group of the enzyme soluble guanylate cyclase to stimulate the conversion of guanosine triphosphate to cyclic guanosine monophosphate. Cyclic guanosine monophosphate then activates protein kinase G which is responsible for the release of intracellular calcium for vasodilation (**Figure 1B**). Endogenously, NO is produced via the action of endothelial nitric oxide synthase (eNOS) to oxidize L-arginine (**Figure 1B**). NO plays a vital role in the regulation of cerebrovascular conductance, and consequently CA (Toda et al., 2009), and the inhibition of eNOS substantially reduces dynamic CA (White et al., 2000). NO bioavailability is also increased independently of the endothelium by the ingestion of nitrate (NO_3^-) rich foods and the subsequent reduction of nitrate to nitrite (NO_2^-), and finally NO (**Figure 1C**). Hypoxia suppresses the oxygen-dependent eNOS pathway but upregulates the production of NO by reduction of nitrate obtained from dietary sources (van Faassen et al., 2009) (**Figure 1B**). Of particular relevance, inhibition of neuronal nitric oxide synthase reduces the haemodynamic response to neural activity in the cerebral cortex. This suggests NO may be pivotal to neurovascular coupling which is essential for cognitive function (Tzeng and Ainslie, 2014) (**Figure 1D**). Indeed, cognitive load impairs dynamic CA (Ogoh et al., 2018), perhaps since they both rely on cerebrovascular capacity for vasodilation. The effect of hypoxia on neurovascular coupling appears dependent on the cognitive domain and responsible brain region, with those regions most susceptible to Alzheimer's-associated decline (e.g., the posterior cingulate cortex) demonstrating a reversal of neurovascular coupling under hypoxia (Rossetti et al., 2020).

Therefore, nitrate supplementation provides a potential non-pharmacological dietary strategy to protect against region-specific vasoconstriction in response to neural activity. Nitrate supplementation may benefit the regulation of cerebrovascular tone to maintain CBF and CA under normoxic and hypoxic conditions, with possible implications for neurovascular coupling and cognitive function.

Dietary nitrate effects on cerebral autoregulation: Recent studies have investigated the effects of dietary nitrate supplementation on dynamic CA (Fan et al., 2019; Horiuchi et al., 2020). Fan et al. (2019) investigated the effect of 7 days of dietary nitrate supplementation (0.1 mmol NO_3^-/kg body weight per day) on dynamic CA assessed by transfer function analysis in healthy adults in normoxia. Compared to the placebo, nitrate supplementation decreased the low-frequency phase parameter of transfer function analysis regardless of sex, indicating a smaller shift in time between the blood pressure and CBF fluctuations, which is indicative of impaired CA. In contrast, nitrate supplementation reduced the low-frequency gain, indicating a smaller change in CBF in proportion to blood pressure changes, and an improvement in dynamic CA. The reduction in gain was observed in men but not women, revealing a possible mediating role of biological sex between dietary nitrate and CA. Fan et al. (2019) also observed that nitrate supplementation improved carbon dioxide (CO_2) reactivity in men, proposing this was due to enhanced cerebrovascular dilatory capacity. In this study, dynamic CA was measured using middle cerebral artery velocity on the assumption that vessel diameter remains constant (Fan et al., 2019). Given NO has established vasodilatory effects, this assumption is questionable. Second, it has been suggested that transfer function analysis-derived estimates of dynamic CA are less sensitive than assessments derived from the classical thigh-cuff method, which imposes a larger and more abrupt change in blood pressure as an autoregulatory stimulus (Tzeng and Ainslie, 2014).

To overcome these issues, we recently investigated the effects of dietary nitrate supplementation on dynamic CA as measured from the combination of the thigh-cuff technique and volumetric CBF assessment by duplex ultrasound, which assesses both blood flow velocity and vessel diameter (Horiuchi et al., 2020). We investigated the effect of 4-day nitrate supplementation (140 mL beetroot juice [8.4 mmol NO_3^-] per day) followed by 60 minutes of normoxia or hypoxia (fraction of inspired oxygen [FiO_2] 13%). As expected, we showed that dynamic CA decreased in hypoxia compared to normoxia. However, compared to placebo nitrate supplementation did not alter dynamic CA in normoxia or hypoxia. Further, nitrate did not affect the carotid artery vessel diameter, blood velocity, or blood flow in either normoxia or hypoxia. In combination with findings from Fan et al. (2019), this suggests that short-term dietary nitrate supplementation in young healthy adults may not benefit CA, even in low oxygen states that have previously been shown to impair CA. However, these findings may be due to the use of experimentally-induced hypoxemia in otherwise healthy young individuals, which is characterized by increased CBF in contrast to the decreased CBF in clinical conditions. The increased CBF at rest may mean there was a mechanical limitation to additional vasodilation with dietary nitrate that may not be present under clinical conditions of ischemia. Future studies of dietary nitrate in these clinical populations are therefore merited. In addition, the role of dietary nitrate in the complex competing effects of oxygen and CO_2 availability on dynamic CA, particularly concerning region-specific effects, warrants further investigation.

Dietary nitrate effects on cognitive function: Early research in older adults highlighted the potential of dietary nitrate to improve cognitive function. Using MRI, Presley et al. (2011) showed that 2 days of a high nitrate diet compared to a low nitrate diet led to increased regional CBF in brain areas known to be involved in executive functioning. To the best of our knowledge, only one study has evaluated the effect of nitrate supplementation on CBF and cognitive function at rest. Wightman et al. (2015) reported that a single dose of beetroot juice (450 mL beetroot juice [5.5 mmol NO_3^-]) improved neurovascular coupling, as assessed by near-infrared spectroscopy, and executive function cognitive task performance in young healthy adults. Other research examining the influence of dietary nitrate on cognition is equivocal. Three days of nitrate supplementation (2 × 70 mL beetroot juice [~9.6 mmol NO_3^-] per day) elicited no change in cognitive performance tests (serial subtractions, rapid visual information processing, number recall) compared to placebo in healthy older adults (Kelly et al., 2013). The authors did observe an increase in NO metabolites, a reduction in blood pressure, and improved oxygen consumption kinetics, but the expected reduction in oxygen cost of walking did not occur. This highlights the time-course of nitrate-induced physiological adaptations is likely different across each specific response. Therefore, the required supplementation duration and dosage cannot be assumed from previous work examining a different physiological phenomenon. In contrast, in older adults with type 2 diabetes, two weeks of nitrate supplementation (250 mL beetroot juice [7.5 mmol NO_3^-] per day) improved simple reaction time compared to placebo, but not other measures of cognitive function including decision reaction time, rapid processing, and memory (Gilchrist et al., 2014). Since these studies did not incorporate measures of cerebral hemodynamics, the mechanism for the potential beneficial effect of nitrate supplementation on cognitive function remains unclear.

More recently, Dobashi et al. (2019) demonstrated in healthy men that 4 days of nitrate supplementation (140 mL beetroot juice [8.4 mmol NO_3^-] per day) did not affect cognitive function at rest or during exercise in hypoxia. That longer nitrate supplementation (or larger daily doses) may be required, is further highlighted by improved cognitive performance during active recovery between sprints in male athletes after 7 days of nitrate supplementation (140 mL beetroot juice [12.8 mmol NO_3^-] per day) (Thompson et al., 2015).

In summary, the findings from acute (single bolus) and short-term (≤ 7 days) supplementation studies are equivocal. However, the only acute study to include assessment of the underlying physiological mechanism provides support that nitrate supplementation may improve cognitive function by improved neurovascular coupling (Wightman et al., 2015). Although the evidence from chronic supplementation studies is lacking, the only longer-term supplementation study (2 weeks)

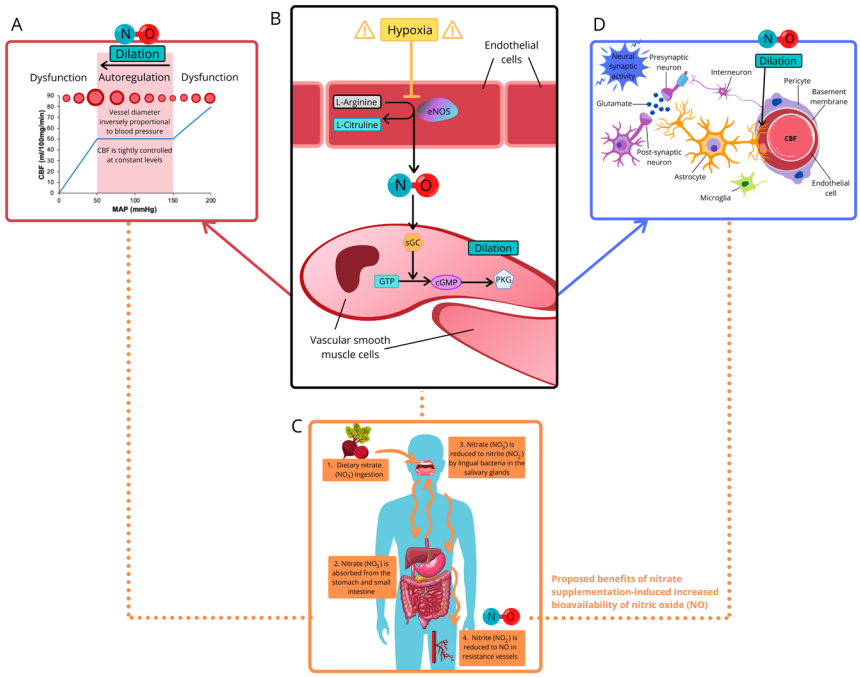

Figure 1 | **Possible mechanism of dietary nitrate benefit on neurovascular coupling and CA through improved NO bioavailability.**
(A) CA maintains cerebral blood flow at constant levels over a range of systemic blood pressures. Endothelium-derived nitric oxide plays a key role in vasodilation of smooth muscle cells, necessary for CA. (B) Hypoxia inhibits the action of endothelial nitric oxide synthase (eNOS) to downregulate the production of endogenous NO, which negatively effects vascular function. (C) Dietary nitrate supplementation may benefit cerebrovascular function through increased NO bioavailability, with implications for CA and (D) neurovascular coupling, necessary for cognitive function. CA: Cerebral autoregulation; CBF: cerebral blood flow; cGMP: cyclic guanosine monophosphate; eNOS: endothelial nitric oxide synthase; GTP: guanosine triphosphate; MAP: mean arterial pressure; NO: nitric oxide; PKG: protein kinase G; SGC: soluble guanylate cyclase.

showed nitrate improved cognitive function in a population known to have poor vascular health (Gilchrist et al., 2014).

Limitations: Nitrate-induced improvements in vascular tone may improve CBF and CA, resulting in the improvement of cognitive function (Wightman et al., 2015). Several well-controlled studies have now been conducted to assess the effect of dietary nitrate supplementation on cerebrovascular function including CA and cognition.

However, the two studies to investigate the effect of nitrate supplementation on CA used relatively short supplementation protocols of up to 1 week (Fan et al., 2019; Horiuchi et al., 2020). Further, the current published evidence for cognitive function benefits is limited, with equivocal findings drawn from a mixture of single-dose and short-term (≤ 7 days) nitrate supplementation. Additionally, convenience sampling in young healthy adults limits our ability to interpret the effectiveness of dietary nitrate in populations that theoretically have the greatest potential to benefit, i.e. those with poor vascular health. Indeed, the only study to investigate cognitive function in a relevant clinical population demonstrated a positive effect (Gilchrist et al., 2014).

With few studies providing appropriate physiological data (e.g., CBF), and differences in the populations studied and cognitive domains assessed, it is difficult to obtain a consensus about the role of dietary nitrate on CA and cognitive function.

Future directions: Fundamentally, whether chronic nitrate supplementation could benefit neurovascular function in populations with cerebral vascular impairments or poor NO bioavailability remains to be investigated. Of note, although NO may potentially have beneficial effects, future studies should also document any harmful effects, for example, dietary nitrate exacerbated headache and cerebral acute mountain sickness symptoms in a recent study (Rossetti et al., 2017). To fully elucidate the effects of dietary nitrate on neurovascular function, future research will need to employ imaging techniques that provide detail of regional brain responses (Rossetti et al., 2020). Further, these complex physiological regulatory systems involve several overlooked processes and control mechanisms including ATP-sensitive K^+ channels, prostaglandins, and reactive oxygen species. These mechanisms may provide alternative avenues for dietary interventions for the rigorous scientist to investigate. The findings of these future investigations will help to confirm or refute the potential of dietary nitrate as a non-pharmacological strategy to benefit neurovascular health.

Conclusion: At present, despite the popularity of dietary nitrate supplementation studies, there is limited evidence to support the use of dietary nitrate supplementation for the maintenance of cerebrovascular and cognitive function.

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